IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of	:	Knipe, et al.
Serial No.	:	08/278,601
Filed	:	July 21, 1994
For	:	Herpesvirus Replication Defective Mutants
Group	:	1645
Examiner	:	Caputa, A.

Assistant Commissioner of Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R.§1.608(b)

I, Robert Finberg, declare:

1. That prior to September 25, 1990, experiments were performed in my laboratory at the Dana Farber Cancer Institute with my knowledge, at the request of and on behalf of David Knipe, a named inventor of the above-captioned application. My curriculum vitae is attached hereto as Appendix A.

2. That the experiments were performed prior to September 25, 1990 by Lien Huong Nguyen who was employed as a post doctorate in my laboratory at the Dana Farber Cancer Institute. Dr. Nguyen is no longer employed by the Dana Farber Cancer Institute.

3. That the following is a factual description of experiments performed by Dr. Nguyen in the United States prior to September 25, 1990.

4. That Dr. Nguyen was requested to perform these experiments by me at David Knipe's request and she performed these experiments with my knowledge.

5. That Appendix B attached hereto are true copies, with dates deleted, of laboratory notebook pages from the notebook of Dr. Nguyen. The notebook was issued by the Dana Farber Cancer Institute and the notebook is still in the possession of the Dana Farber Cancer Institute. The dates deleted from the notebook pages are dates prior to September 25, 1990.

6. That I recognize the handwriting on the notebook pages as the handwriting of Dr. Nguyen.

7. That the notebooks were maintained in conjunction with the performance of the experiments performed by Dr. Nguyen in the United States before September 25, 1990.

8. That based on personal knowledge, Dr. Nguyen demonstrated, in the United States prior to September 25, 1990, that two different mutant herpesviruses protected mice against a lethal dose of wild-type herpesvirus, HSVmP. The mutant herpesviruses that provided such protection were not capable of producing additional virus in cells other than cells that complemented the defective genes. In particular, the mutated viruses used in these experiments consisted of one herpesvirus containing a deletion mutation in the gene that expresses ICP8, known as mutant d301; and the other herpesvirus containing a nonsense insertion mutation in the gene expressing ICP27, known as mutant n504R.

9. That the mutant herpesviruses were obtained from David Knipe with the understanding that my laboratory would perform experiments to demonstrate that such mutant viruses were protective against wild-type herpesvirus.

10. That the experiments performed by Dr. Nguyen in the United States prior to September 25, 1990 were as follows:

> 10^6 pfu of replication-defective viruses, those containing mutations in the genes encoding ICP8 or ICP27, were injected into mice, and then challenged with a lethal dose of 10^8 pfu live wild-type HSV-1 virus. The mice that received the mutants had 100 %

survival rates whereas the control mice that did not receive mutant virus had a 10 % survival rate. Thus the experiments demonstrated that replication defective mutants of

HSV-1 induced immunity in mice injected with the mutant viruses and protected against lethal infection whereas the majority of mice injected with control material and subsequently challenged with wild type virus, died.

11. That the following correlates the above-described experiment to the notebook pages provided in Appendix B:

A. Female Balb/c mice, 5 to 7 weeks of age, were used for the experiment. These mice were injected intraperitoneally with the viruses or control samples.

This is stated on page **HOO3388**:

second line "injection mice with";

third line right side of page near the margin "n=8 Balb";

just below the middle of the page on the right across from the number ② "n=8Balb mice".

and on page **HOO3497** first and second lines where it is written

"① Balb mice.... ♀. 5-7 weeks by _____ ."

"n" refers to the number of mice in the group.

B. Viruses used in the experiment were obtained from the laboratory of Dr. David Knipe.

This is stated on page **HOO3388**

top line on the left "All virus received from Dr. David Knipe _____";

fifth line: "ICP8 stock d301 _____ received from Dr. David Knipe's Lab (Kay) on day _____."

The titer of the viruses was $1.7x10^9$ pfu/cc for the ICP8 mutant virus page **HOO3388** 8th line and $4x10^8$ pfu for ICP237 (n504R) on line midpage in paragraph ②.

"ICP8" refers to the replication defective mutant virus containing a mutation in the gene encoding ICP8, termed d301; "ICP27" refers to the replication defective mutant virus containing a mutation in the ICP27 gene, termed n504R.

C. The mutant viruses were diluted to 10^6 pfu/cc in an injection volume per mouse of .5cc.

This is shown on page **HOO3388**

par.① 6th line "Need 10^6 pfu/cc. so do a 1:1700 dilution that means :100λ in 170000 = 170cc or: 100λ (virus stock) in 85cc PBS and injection of .5cc"

par.② 2nd line "Need 10^6 pfu/cc: So do a 1: 4 10^2 Dilution that means 100λ in 40000 = 40cc. PBS or 100λ (virus) in 20cc and inject 0.5cc."

D. Groups of eight mice were injected with 10^6 pfu of each of the replication defective mutants ICP8 (d301) and ICP27 (n504R). Control mice (group of 9 mice) were injected with PBS.

This is shown on page **HOO3388**

third line right side of page near the margin "n=8 Balb";

just below the middle of the page on the right across from the number ② "n=8Balb mice".

and on page **HOO3497**

par. ① "Balb mice....♀. 5-7 weeks by _____ ."

" _____ : challenged with

106pfu HSV ICP8 n=8 (2)

106pfu HSV ICP27 n=8 (3)

and n=9 (4)

PBS as control"

E. Six weeks plus 5 days later, all the mice were challenged with 10^8 pfu of a virulent wild-type HSV-1 strain , HSV-1 (mP).

This is shown on notebook page **HOO3497**:

mid page: "Date deleted : challenge with 10^8 pfu HSV-mP."

F. Mortality was determined 10 days post challenge with HSV-1mP. As reported on notebook page HOO3487, two mice each from the groups injected with mutant virus were removed for proliferation assay studies leaving six mice per group. The mice which

had been inoculated with the mutants ICP8 (d301) or ICP27 (n504), 0 (zero) mice of six died; 1/9 control (PBS injected) mice survived, that is 8 out 9 mice died.

This is shown on the bottom half of page **HOO3497** as follows:

"Mortality:

in 10 days

(2) (ICP8) 0 died.

(3) (ICP27) 0 died.

(4) 8 died from 9. (control)"

12. That I hereby declare that all statements made herein are true, and all statements made on information and belief are believed to be true, and further that all statements were made with the knowledge that any willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-identified application or any patent issued thereon.

Date: 9/19/97

Robert Finberg

CURRICULUM VITAE

Name: Robert William Finberg

Address: 48 Spring Lane, Canton, Massachusetts 02021

Place of Birth: Baltimore, Maryland

Education:

1971	A.B.	University of Chicago
1974	M.D.	Albert Einstein College of Medicine
1996	M.A.	(Hon.) Harvard University

Postdoctoral Training:

Internship and Residencies:

1974-1975	Intern in Medicine, Bellevue Hospital, New York
1975-1976	Junior Resident in Medicine, Bellevue Hospital
1976-1977	Senior Resident in Medicine, Bellevue Hospital
1979-1980	Fourth Year Resident Physician, Peter Bent Brigham Hospital, Boston, MA

Clinical and Research Fellowships:

1977-1978	Research Fellow in Pathology, Harvard Medical School, Boston, MA
1978-1979	Research Fellow in Medicine, Harvard Medical School
1978-1979	Research/Clinical Fellow in Medicine, Peter Bent Brigham Hospital
1979-1980	Clinical Fellow in Medicine, Harvard Medical School

Licensure and Certification:

1976	Massachusetts License Registration No. 40199
1976	American Board of Internal Medicine, Candidate No. 058714
1980	Board Certified - Infectious Diseases, Candidate No. 058714

Academic Appointments:

1980-1984	Assistant Professor of Medicine, Harvard Medical School
1985-1995	Associate Professor of Medicine, Harvard Medical School
1996-Present	Professor of Medicine, Harvard Medical School

Hospital Appointments:

1980-1982	Junior Associate in Medicine, Brigham & Women's Hospital, Boston, MA
1980-1984	Assistant Physician, Chief of Infectious Diseases, Dana-Farber Cancer Institute, Boston, MA
1982-	Associate Physician, Brigham & Women's Hospital
1985-1986	Courtesy Staff, The Children's Hospital, Boston, MA
1985-1992	Associate Physician, Chief of Infectious Diseases, Dana-Farber Cancer Institute
1986-	Staff Physician, The Children's Hospital
1992-1995	Associate Professor of Medicine, Chief of Infectious Diseases, Dana-Farber Cancer Institute
1996-Present	Professor of Medicine, Chief of Infectious Diseases, Dana-Farber Cancer Institute

Awards and Honors:

1973	Alpha Omega Alpha
1980-1983	Hartford Foundation Award for the support of faculty in scientific research
1983-1988	Scholar of the Leukemia Society

Major Committee Assignments:

Government:

1984	Special Reviewer, Experimental Immunology Study Section
1985-1989	Regular Reviewer, Experimental Immunology Study Section
1990-1995	Secretarial appointee, Department of Veterans Affairs, Medical Research Service, Career Development Committee
1991-1994	AIDS and Special Virology and Vaccines Ad Hoc Committees
1995-7	Special Reviewer, Immunobiology Study Section

Harvard Medical School, Graduate Student Supervision:

1980-	Member, Committee on Virology
1983-	Member, Committee on Immunology

Memberships in Professional Societies:

1974-	American Association for the Advancement of Science
1978-	American Society of Microbiology
1979-	American Association of Immunologists
1979-	American College of Physicians
1980-	American Federation for Clinical Research
1982-	Infectious Disease Society of America, Fellow
1985-	American Society for Clinical Investigation
1987-	Pediatric Infectious Disease Society
1988-	Clinical Immunological Society
1992-	Immunocompromised Host Society

Editorial Boards:

1982-1985	Infection and Immunity
1982-1995	Infectious Disease Practice
1984-	Journal of Immunology
1984-	Survey of Immunologic Research

Major Research and Clinical Interests:

1. The importance of T cells in mammalian responses to viruses
2. The regulatory role of T cells in response to bacterial infections
3. GPI-Anchored Proteins as Signal transduction molecules
4. HIV-1 - CD4 interactions
5. Picornavirus receptors
6. Infections in immunocompromised patients

Research Funding Information

Active

1992-2000 NIH, RO1AI31628 PI
Cell Surface Proteins Involved in Echovirus Attachment

1995-1998 NIH PO1 A137963-01 Co-PI
Mechanisms Involved in the Generation of Protective Immunity

1989-1999 NIH 2P30 AI28691-06 Co-PI
AIDS Center Support Grant

1997-2002 NIH, RO1 AI 39576-01 Co-PI
Pathogenic Mechanisms of Anaerobes in Sepsis

1995-1998 IDF International: Juvenile Diabetes PI
A Virus Induced Autoimmune Disease

1998-2001 Novartis Drug Discovery Program PI
Role of Bcl-2/x viral homologues in epithelial maligancies

Expired

1996-1997 Aroncx: A randomized trial of liposomal nystatin versus amphotericin B PI

1994-1996 Fujisawa: A randomized trial of Ambisome versus amphotericin B PI

1995-1996 Omnibus Solicitation: PHS SBIR, PHS 95-3 PI
Virus Inactivation in Blood Using Microwave Heating

1995-1996 Women's Breast Cancer Program PI
G Proteins and GPI-Anchored Surface Proteins in Tumor Cells

1995-1997 Fujisawa, USA PI
Trial: Ambisome vs. Amphotericin B

1992-1996	NIH, P01 A133087 Co-PI Clinical and Laboratory Studies of PID
1994-1995	DFCI Drug Discovery Program PI D2: A Signal Transducing Molecule Present on Tumor Cells
1994-1995	Barr Program Small Grants PI Characterization of Receptor Proteins for Diabetogenic Viruses
1991-1994	NIH, NO1-DE-12585 (subcontract) Co-PI Role of Mononuclear Phagocytes in Opportunistic Infections of Oral Mucosa and Other Tissues in AIDS Patients
1992-1993	Seragen, Inc. PI IL-2 toxin and immune responses
1992-1995	American Heart Association #92013820 PI Cell Surface Proteins Involved in Echovirus Attachment
1983-1993	NIH, RO1CA3479 PI Animal Models of AIDS
1982-1993	NIH, RO1AI20382 PI Cell Mediated Immune Response to Murine Viruses
1990-1993	NIH 1R01AI29657-02 Opioids and Opiates: T Cell Motility
1989-1990	Massachusetts Mutual Life Insurance Company PI A Study on the Measurement of Immune Responses to the AIDS Virus in Children
1987-1990	DAMD-87-C-7151 PI Analysis of the Human T cell Response to HTLV-III
1990-1991	DFCI Center for AIDS Research PI Resistance of Human T Cells to HIV-1 Infection
1989-1990	Seragen, Inc. PI Effects of IL-2 Toxin on HIV-1 Infection of Cells
1990-1991	Seragen, Inc. PI Use of DAB486 IL-2 to Eliminate HIV-1 Infected Cells
1990-1991	DFCI Center for AIDS Research PI CPF: An HIV-1 Binding Peptide
1984-1989	5R01 AI20541 PI Immune Regulation by Cytotoxic T Cell Clones
1983-1988	LSA Scholar PI Use of T Cell Hybridomas in Infection
1986-1987	AmFAR, Grant No. 00104 PI Analysis of T-Cell Responses to AIDS

1985-1987	Serono Laboratories PI Effect of Thymic-derived Lymphokines on T Cell Responses and Infections in Bone Marrow Transplant Patients
1985-1986	Whittaker Foundation PI Cloning and Genomic Analysis of a Receptor Molecular from an Ag Specific T Cell Hybridoma
1983-1985	Biogen, Inc. PI Investigation of T cells in Patients with AIDS
1980-1985	Hartford Foundation PI Award for Junior Faculty
1980-1992	NIH, 5R01 AI20382 PI Cell Mediated Immune Response to Murine Viruses

Pending

1998-2003	NIH, R01 GM57520 PI LPS Mediated Endotoxic Shock: Mechanisms of Pathogenesis
1997-1999	Pfizer, Inc A randomised trial of Voriconazole versus amphotericin B PI

Principal Clinical and Hospital Service Responsibilities:

1980-	Attending in Medicine and Infectious Diseases, Brigham & Women's Hospital
1980-	Consultant in Infectious Diseases, Children's Hospital, Boston, MA
1982-	Chief, Infectious Diseases, Dana-Farber Cancer Institute

Teaching Experience:

Classroom:

1980, 83-84	Pathophysiology 902 conference leader, Harvard Medical School
1981-1982	Immunology 700 lecturer, Harvard Medical School
1986	Tutor, New Pathway, Harvard Medical School
1987	Lecturer, Immunobiology 204, Harvard School of Public Health, Boston, MA
1987-1990	Tutor, Identify and Defense, New Pathway, Harvard Medical School
1990-1992	Case Coordinator for Immunology and Microbiology, Harvard Medical School
1991-1992	Lecturer, Virology 314, Harvard Medical School
1991-	Member, Advanced Basic Science Committee
1993-	Senior Fellow for ABS, Harvard Medical School
1992-	Modern Medical Microbe Hunters (IN505.J), course director
1992-	Interactions of Viruses with Mamalian Cells (ME551.5) , course director

Clinical:

1980-1981	Consult visit in Infectious Disease, Brigham & Women's Hospital
1981-	Consult visit in Infectious Disease, Dana-Farber Cancer Institute
1981-	Ward visit in Medicine, Brigham & Women's Hospital
1983-	Consult visit in Infectious Disease, The Children's Hospital

BIBLIOGRAPHY

Original Reports:

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2. Burakoff SJ, Finberg R, Glimcher L, Lemonnier F, Benacerraf B, Cantor H. The biologic significance of alloreactivity. The ontogeny of T-cell sets specific for alloantigens or modified self antigens. J Exp Med. 1978;148:1414-22.

3. Finberg R, Mescher M, Burakoff SJ. The induction of virus-specific cytotoxic T lymphocytes with solubilized viral and membrane proteins. J Exp Med. 1978;148:1620-7.

4. Finberg R, Burakoff SJ, Cantor H, Benacerraf B. Biological significance of alloreactivity: T cells stimulated by Sendai virus-coated syngeneic cells specifically lyse allogeneic target cells. Proc Natl Acad Sci. USA. 1978;75:5145-9.

5. Finberg R, Weiner HL, Fields BN, Benacerraf B, Burakoff SJ. Generation of cytolytic T lymphocytes after reovirus infection: role of *S1* gene. Proc Natl Acad Sci USA. 1979;76:442-6.

6. Finberg R, Greene MI, Benacerraf B, Burakoff SJ. The cytolytic T lymphocyte response to trinitrophenyl-modified syngeneic cells. I. Evidence for antigen-specific helper T cells. J Immunol. 1979;123:1205-9.

7. Finberg R, Burakoff SJ, Benacerraf B, Greene MI. The cytolytic T lymphocyte response to trinitrophenyl-modified syngeneic cells. II. Evidence for antigen-specific suppressor T cells J Immunol. 1979; 123:1210-4.

8. Finberg R, Cantor H, Benacerraf B, Burakoff S. The origins of alloreactivity: differentiation of prekiller cells to viral infection results in alloreactive cytolytic T lymphocytes. J Immunol. 1980;124(4):1858-60.

9. Burakoff SJ, Reiss CS, Finberg R, Mescher MF. Cell-mediated immunity to viral glycoproteins. Rev Infec Dis. 1980;2(1):62-78.

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11. Finberg R, Weiner HL, Burakoff SJ, Fields BN. Type-specific reovirus antiserum blocks the cytotoxic T-cell-target cell interaction: evidence for the association of the viral hemagglutinin of a nonenveloped virus with the cell surface. Infect Immun. 1981;31:646-9.

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15. Takaoki M, Sy M-S, Whitaker B, Nepom J, Finberg R, Germain RN, Nisonoff A, Benacerraf B, Greene MI. Biologic activity of an idiotype-bearing suppressor T cell factor produced by a long-term T cell hybridoma. J Immunol. 1982;128(1):49-53.

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20. Finberg R, Spriggs DR, Fields BN. Host immune response to reovirus: CTL recognize the major neutralization domain of the viral hemagglutinin. J Immunol. 1982;129:2235-8.

21. Takaoki M, Sy M-S, Tominaga A, Lowy A, Tsurufuji M, Finberg R, Benacerraf B, Greene MI. I-J restricted interactions in the generation of azobenzenearsonate-specific suppressor T cells. J Exp Med. 1982;156:1325-34.

22. Letvin NL, Kauffman RS, Finberg R. An adherent cell lyses virus-infected targets: characterization, activation, and fine specificity of the cytotoxic cell. J Immunol. 1982;129(6):2396-401.

23. Ertl HCJ, Greene MI, Noseworthy JH, Fields BN, Nepom JT, Spriggs DR, Finberg RW. Identification of idiotypic receptors on reovirus-specific cytolytic T cells. Proc Natl Acad Sci. USA. 1982;79:7479-83.

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25. Ertl HCJ, Brown EG, Finberg RW. Sendai virus-specific T cell clones. II. Induction of interferon production by Sendai virus-specific T helper cell clones. Eur J Immunol. 1982;12:1051-3.

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28. Wolf JL, Kauffman RS, Finberg R, Dambrauskas R, Fields BN, Trier JS. Determinants of reovirus interaction with the intestinal M cells and absorptive cells of murine intestine. Gastroenterology. 1983;85:291-300.

29. Sy M-S, Tsurufuji M, Finberg R, Benacerraf B. Effect of vesicular stomatitis virus (VSV) infection on the development and regulation of T cell-mediated immune response. J Immunol.

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31. Kauffman RS, Lee S, Finberg R. Cytolytic T-cell mediated lysis of reovirus-infected cells: requirements for infectious virus, viral particles, and viral proteins in infected target cells. Virology. 1983;131:265-73.

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35. Ertl HCJ, Finberg RW. Sendai virus-specific T-cell clones: induction of cytolytic T cells by an anti-idiotypic antibody directed against a helper T-cell clone. Proc Natl Acad Sci .USA. 1984;81:2850-4.

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43. Sharpe AH, Gaulton GN, Ertl HCJ, Finberg RW, McDade KK, Fields BN, Greene MI. Cell receptors for the mammalian reovirus. IV. Reovirus-specific cytolytic T cell lines that have idiotypic receptors recognize anti-idiotypic B cell hybridomas. J Immunol. 1985;134:2702-6.

44. Zaleznik DF, Finberg RW, Shapiro ME, Onderdonk AB, Kasper DL. A soluble suppressor T cell factor protects against experimental intraabdominal abscesses. J Clin Invest. 1985;75:1023-7.

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48. Ertl HCJ, Skinner MA, Finberg RW. Induction of anti-viral immunity by an anti-idiotypic antibody directed to a Sendai virus specific T helper cell clone. Intern Rev Immun. 1986;1:41-65.

49. Shapiro ME, Kasper DL, Zaleznik DF, Spriggs S, Onderdonk AB, Finberg RW. Cellular control of abscess formation: role of T cells in the regulation of abscesses formed in response to *Bacteroides fragilis*. J Immunol. 1986;137:341-6.

50. Skinner MA, Finberg RW, Ertl HCJ. Regulation of cytoxic lymphocyte precursors. II. The effect of interleukin-2 and interferon-gamma on the apparent specificity of effector cells. Cell Immunol. 1986;100:239-46.

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57. Cohen JI, Deresiewicz RL, Finberg RW. Fever, hypotension, and pulmonary infiltrates in a young Haitian man [clinical conference.] Rev Infect Dis. 1988;10:1052-60.

58. Talcott JA, Finberg R, Mayer RJ, Goldman L. The medical course of cancer patients with fever and neutropenia. Arch Intern Med. 1988;148:2561-8.

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62. Hom RC, Soman G, Finberg R. Trojan horse lymphocytes : a vesicular stomatitis virus specific T cell clone lyses target cells by carrying virus. J Virol. 1989;63;4157-64.

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viel glück ! All virus received from Dr David Knipe.

(A) injection mice with:

(1) ICP8: 10^6 pfu $n = 8$ Balb

ICP8 stock d301 ( )
received from Dr David Knipe's
Lab (Kay) on day

1.7 10^9 pfu / cc = 17 . 10^8 pfu / cc.
Need 10^6 pfu / cc.
so do : a 1 : 1700 Dilution
that means : 100λ in 170000 = 170 cc
(virus stock)
or : 100λ in 85 cc PBS
and injection of .5 cc

(2) ICP27: (-n 504 R) 4 x 10^8 pfu/cc
ICP27 (n504 4.10^8)
$n = 8$
Need 10^6 pfu / cc : Balb mice.

So do : a 1 : 4 10^2 Dilution
that means 100 λ in 40000 = 40 cc. PBS
(virus)
or 100λ in 20 cc
and inject 0.5 cc.

(3) ICP4: (received from Dr. Neal de Luca)
5.5. 10^8 pfu / cc
≙ Need 100λ than do a
1 : 5.5. 10^2 = 550 Dilution

Experiment:

(1) Balb mice / c Antac ♀.
5-7 weeks by

: challenged c̄

10^6 pfu	HSV	ICP_4	$n = 8$	(1)
10^6 pfu	HSV	ICP_8	$n = 8$	(2)
10^6 pfu	HSV	ICP_{27}	$n = 8$	(3)
and			$n = 9$	(4)
PBS	as	Control		

1. Bleeding
2. Bleeding

: Challenge c̄ 10^8 pfu

HSV - mp.
Mortality:
in 10 days

(1) (ICP_4) 2 died / from 6. (2 for protection assay) — total

(2) (ICP_8) ∅ died.

(3) (ICP_{27}) ∅ died.

(4) 8 died from 9 (Control)